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FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER: 3501-1002 U.S. APPLN NO. (If known, see 37 CFR 1.5) 10/030920
INTERNATIONAL APPLICATION NO.: PCT/FI00/00624	INTERNATIONAL FILING DATE: 6 JULY 2000	PRIORITY DATE CLAIMED: 12 JULY 1999

TITLE OF INVENTION: METHOD OF PURIFYING WATER, SUITABLE BACTERIA FOR THE METHOD AND USE THEREOF

APPLICANT(S) FOR DO/EO/US: Jussi UOTILA and Gennadi ZAITSEV

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. [X] has been transmitted by the International Bureau. (see attached copy of PCT/IB/308)
   - c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   - a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. [ ] have been transmitted by the International Bureau.
   - c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   - d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Item 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

International Search Report
PCT/IPEA/409
Application Data Sheet
PCT/IB/308
Abstract of the Disclosure on a Separate Sheet

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U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 10/030920	INTERNATIONAL APPLICATION NO. PCT/FI00/00624	ATTORNEY'S DOCKET NO. 3501-1002

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR1.482) nor international search fee (37 CFR1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .... $ 1,040.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .... $ 890.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .... $ 740.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .... $ 710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) .... $ 100.00

				CALCULATIONS	PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	19 - 20 =	0	X $18.00	$	
Independent claims	10 - 3 =	7	X $84.00	$ 588.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ $280.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 1,478.00	
Reduction of ½, if applicant is entitled to Small Entity status under 37 CFR 1.27. +				$ 739.00	
SUBTOTAL =				$ 739.00	
Processing fee of $130 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR1.492(f)).				$	
TOTAL NATIONAL FEE =				$ 739.00	
Fee for recording the enclosed assignment (37 CFR1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 739.00	
				Amount to be refunded:	
				charged:	

a. [X] A check in the amount of $ <u>739.00</u> to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. **25-0120** in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. **25-0120**. A duplicate copy of this sheet is enclosed.

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January 14, 2002

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PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Jussi UOTILA et al.

Serial No. (unknown)

Filed herewith

METHOD OF PURIFYING WATER,
SUITABLE BACTERIA FOR THE
METHOD AND USE THEREOF

PRELIMINARY AMENDMENT

Commissioner for Patents

Washington, D.C. 20231

Sir:

Prior to the first Official Action and calculation of the filing fee, please substitute Claims 1-21 as originally filed, with Claims 1-19 as filed in the Article 34 amendment of September 6, 2001. The pages containing Claims 1-19 are marked "AMENDED SHEET" and are attached hereto. Following the insertion of Claims 1-19, please amend these claims as follows:

IN THE SPECIFICATION:

Please replace the paragraph beginning at page 18, line 21, with the following rewritten paragraph:

--Waste water from a coating metal industry plant was purified by a system whose effective treatment part comprised six anaerobic and twelve aerobic tanks. The bacteria DT-1, DT-2 and DT-5, which were immobilized on a carrier attached by nets, were added to all anaerobic and aerobic tanks. Each tank

held 2 l. The entire system comprised 23 tanks whose total volume was 70 l, the tanks being interconnected in the following order: six anaerobic tanks (effective treatment volume), one sedimentation tank, six aerobic tanks (effective treatment volume), one sedimentation tank, six aerobic tanks (effective treatment volume), and two tanks for calcium chloride and sodium hydroxide treatments to precipitate the biomass and heavy metals. Before the treatment, the original waste water was diluted five times by gray water. After the dilution, mineral salts were added as follows: NH_4^+ 2 - 10 mg/l, NO_3^- 5 - 20 mg/l, Mg^{2+} 2 - 10 mg/l, Ca^{2+} 0.5 - 2 mg/l, SO_4^{2-} 1 - 10 mg/l and PO_4^{3-} 2 - 20 mg/l. The temperature was 20 - 35°C and the flow rate 12 l of water per 24 hours. The results are shown in Table 5.--

IN THE CLAIMS:

Amend the claims as follows:

--3. (amended) A method as claimed in claim 1, **characterized** in that necessary biomass for the purification is produced in a non-sterilized growth medium comprising tap water and about 0.5 - 4 g/l of soap.--

--14. (amended) Use of a bacterial mixed population as claimed in claim 12 in purifying waste water.--

R E M A R K S

The above changes in the specification and claims merely place this national phase application in the same condition as it was during Chapter II of the international phase, with the multiple dependencies being removed. Following entry of this amendment by substitution of the pages, only claims 1-19 remain pending in this application.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE".

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows:

3. (amended) A method as claimed in claim 1~~ or 2~~, **characterized** in that necessary biomass for the purification is produced in a non-sterilized growth medium comprising tap water and about 0.5 - 4 g/l of soap.

14. (amended) Use of a bacterial mixed population as claimed in claim 12~~or 13~~ in purifying waste water.

CLAIMS

1. A method of purifying waste water, **characterized** in that the water is biologically purified by a mixed population comprising the microorganisms *Bacillus* sp. DT-1 having the deposit number DSM 12560, *Pseudomonas azelaica* DT-2 having the deposit number DSM 12561, and *Rhizobium* sp. DT-5 having the deposit number DSM 12562, or progeny thereof.

2. A method as claimed in claim 1, **characterized** by purifying seep water, grey water, black water, industrial waste water and waste water from laundries.

3. A method as claimed in claim 1 or 2, **characterized** in that necessary biomass for the purification is produced in a non-sterilized growth medium comprising tap water and about 0.5 - 4 g/l of soap.

4. A method as claimed in claim 1, **characterized** in that the water is also purified by one or more microorganisms from the group *Pseudomonas azelaica* DT-6 having the deposit number DSM 13516, *Azospirillium* sp. DT-10 having the deposit number DSM 13517, *Ancylobacter aquaticus* DT-12 having the deposit number DSM 13518, and *Xanthobacter* sp. DT-13 having the deposit number DSM 13519, and progeny thereof.

5. *Bacillus* sp. DT-1 having the deposit number DSM 12560 and progeny thereof.

6. *Pseudomonas azelaica* DT-2 having the deposit number DSM 12561 and progeny thereof.

7. *Rhizobium* sp. DT-5 having the deposit number DSM 12562 and progeny thereof.

8. *Pseudomonas azelaica* DT-6 having the deposit number DSM 13516 and progeny thereof.

9. *Azospirillium* sp. DT-10 having the deposit number DSM 13517 and progeny thereof.

10. *Ancylobacter aquaticus* DT-12 having the deposit number DSM 13518 and progeny thereof.

11. *Xanthobacter* sp. DT-13 having the deposit number DSM 13519 and progeny thereof.

12. A bacterial mixed population, **characterized** by comprising *Bacillus* sp. DT-1 having the deposit number DSM 12560,

Pseudomonas azelaica DT-2 having the deposit number DSM 12561, and/or *Rhizobium* sp. DT-5 having the deposit number DSM 12562, and progeny thereof.

13. A bacterial mixed population as claimed in claim 12, **characterized** by further comprising *Pseudomonas azelaica* DT-6 having the deposit number DSM 13516, *Azospirillium* sp. DT-10 having the deposit number DSM 13517, *Ancylobacter aquaticus* DT-12 having the deposit number DSM 13518, and/or *Xanthobacter* sp. DT-13 having the deposit number DSM 13519, and progeny thereof.

14. Use of a bacterial mixed population as claimed in claim 12 or 13 in purifying waste water.

15. A bioreactor, **characterized** by comprising the microorganisms *Bacillus* sp. DT-1 having the deposit number DSM 12560, *Pseudomonas azelaica* DT-2 having the deposit number DSM 12561, and *Rhizobium* sp. DT-5 having the deposit number DSM 12562, or progeny thereof.

16. A bioreactor as claimed in claim 15, **characterized** by further comprising one or more microorganisms from the group *Pseudomonas azelaica* DT-6 having the deposit number DSM 13516, *Azospirillium* sp. DT-10 having the deposit number DSM 13517, *Ancylobacter aquaticus* DT-12 having the deposit number DSM 13518, and *Xanthobacter* sp. DT-13 having the deposit number DSM 13519, or progeny thereof.

17. A bioreactor as claimed in claim 16, **characterized** by comprising all said seven bacterial strains.

18. A bioreactor as claimed in claim 15, **characterized** by comprising one or more separating walls arranged so as to force water to circulate in the reactor.

19. A bioreactor as claimed in claim 18, **characterized** in that the bacteria are immobilized on a plastic carrier medium whose specific density is about 0.8 g/cm^3.

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METHOD OF PURIFYING WATER, SUITABLE BACTERIA FOR THE METHOD AND USE THEREOF

FIELD OF THE INVENTION

The invention relates to a method of purifying waste water biologically, and to bacteria and a mixed bacterial population suitable for the method and the use thereof. The invention further relates to a bioreactor comprising said bacteria or mixed population.

BACKGROUND OF THE INVENTION

Conventionally, water can be purified both by physical and chemical means, for example by sedimentation, filtration or flocculation (WO94/5866 and WO88/5334). In order to remove organic compounds and other compounds that are difficult to purify it is also preferable to use so-called biological purification wherein the water to be purified is brought into contact with microorganisms that decompose pollution agents. Biological water treatment methods are suited for use both in conventional water treatment plants and industrial waste water treatment plants. Biological water treatment has also been tested in systems where water is recycled (FI 964141). Biological water treatment is also needed to purify seep water of a dump, for example, before the seep water is discharged into the environment.

The biological purifying method is, however, more difficult to control than the physical or chemical purifying methods. Firstly, microorganisms to decompose pollution agents must be found. Secondly, the microorganisms must be capable of easily surviving and reproducing under conditions during the water treatment process. In other words, the microorganisms used for purifying water must be competitive ones so as to prevent other organisms in the water from overruling. In addition, the microorganisms used for purifying water must not be sensitive to the changes in their environment that often occur during water treatment processes when the load varies.

Many kinds of microorganisms have been used for purifying water, including bacteria and protozoa, such as the ciliates. Bacteria that have often been used include species of the *Pseudomonas* genus, but also members of the *Alcagenes*, *Acinetobacter* or *Rhodococcus* genera are often used. Mixed populations, some identified and some unidentified, comprising a great number of different microorganisms are often used. Aerobic or facultative microorganisms are best suited to purifying water, in which case it is appropriate to

pump air into the water to be purified so as to make the purification process more efficient.

When microorganisms are cultivated, the growth medium should normally be sterilized so as to prevent the cultivation from becoming contaminated by external organisms. Since large amounts of water are processed while purifying waste water, the amount of necessary biomass for the biological purification is also large. To produce such biomass under sterile conditions is both laborious and expensive; hence, it would be most desirable if the biomass could be produced under non-sterile conditions without any danger of becoming contaminated. The present invention now provides a novel fermentation technology with no need to sterilize. This is possible when microorganisms particularly suitable for the method are used and these microorganisms are fed on nutrients suitable for them.

SUMMARY OF THE INVENTION

The present invention relates to microorganisms that are surprisingly well suited to biological purification of waste water. These microorganisms meet particularly well the aforementioned requirements set for microorganisms suitable for the biological purification of water. In addition, the microorganisms of the invention are so specific that their biomass can be produced under non-sterile conditions by using a growth medium where other microorganisms are unable to compete. This enables large savings in the costs and energy consumption of a biological water purification process, the purification results also being excellent. Water purified according to the invention is even recyclable.

The invention thus relates to the bacteria *Bacillus* sp. DT-1 having the deposit number DSM 12560 and progeny thereof, *Pseudomonas* sp. DT-2, subsequently identified as *Pseudomonas azelaica* having the deposit number DSM 12561 and progeny thereof, and the former *Pseudomonas* sp. now being *Rhizobium* sp. and having the deposit number DSM 12562 and progeny thereof. Later 16S rDNA analyses have shown that this bacterium most closely resembles the members of the *Rhizobium* genus, so hereafter, it will be considered as one of them. The invention further relates to the following bacterial strains promoting water purification: *Pseudomonas azelaica* DT-6 having the deposit number DSM 13516, *Azospirillium* sp. DT-10 having the deposit number DSM 13517, *Ancylobacter aquaticus* DT-12 having the deposit number

DSM 13518, and *Xanthobacter* sp. DT-13 having the deposit number DSM 13519, and progeny thereof. DSM 12560 - 12562 have been deposited at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH on 1 December 1998, and DSM 13516 - 13519 on 29 May 2000.

The invention further relates to a bacterial mixed population characterized by comprising the bacterium *Bacillus* sp. DT-1 having the deposit number DSM 12560, *Pseudomonas azelaica* DT-2 having the deposit number DSM 12561, and/or *Rhizobium* sp. DT-5 having the deposit number DSM 12562, and progeny thereof.

The invention further relates to the use of the aforementioned bacteria or bacterial mixed populations in waste water treatment and to a method of purifying waste water, characterized by purifying water biologically by microorganisms belonging to the group *Bacillus* sp. DT-1 having the deposit number DSM 12560, *Pseudomonas azelaica* DT-2 having the deposit number DSM 12561, and *Rhizobium* sp. DT-5 having the deposit number DSM 12562, and progeny thereof.

The invention further relates to a bioreactor characterized by comprising microorganisms belonging to the group *Bacillus* sp. DT-1 having the deposit number DSM 12560, *Pseudomonas azelaica* DT-2 having the deposit number DSM 12561, and *Rhizobium* sp. DT-5 having the deposit number DSM 12562, and progeny thereof. A bioreactor is a reactor in which a biological purification process is conducted.

DRAWINGS

Figure 1 schematically shows a purification system for seep water,
Figure 2a shows a profile of the fatty acids of bacterial strain DT-1,
Figure 2b is a print of a fatty acid analysis of bacterial strain DT-1,
Figure 3a shows a profile of the fatty acids of bacterial strain DT-2,
Figure 3b is a print of a fatty acid analysis of bacterial strain DT-2,
Figure 4 is a print of a fatty acid analysis of bacterial strain DT-5,
Figure 5 is a print of a fatty acid analysis of bacterial strain DT-6,
Figure 6 is a print of a fatty acid analysis of bacterial strain DT-10,
Figure 7 is a print of a fatty acid analysis of bacterial strain DT-12, and
Figure 8 is a print of a fatty acid analysis of bacterial strain DT-13.

DETAILED DESCRIPTION OF THE INVENTION

Microorganisms growing in a soap mixture were enriched from waste water of an industrial plant and then adapted by cultivating them in a bioreactor comprising waste water from a dump. Three bacterial strains were thus isolated that were superior to the others. Said bacterial strains are *Bacillus* sp. DT-1 having the deposit number DSM 12560, *Pseudomonas azelaica* DT-2 having the deposit number DSM 12561 and *Rhizobium* sp. DT-5 having the deposit number DSM 12562. These bacteria can be cultivated in tap water comprising about 1 - 4 g/l of soap. Extremely few microorganisms can actively grow under such conditions; therefore, this growth medium needs not be sterilized when biomass of said three bacteria is being produced. The strains tolerate as high amounts of soap as about 40 g/l. They grow best in a soap content of about 0.3 - 0.5 g/l.

In addition to being capable of growing in a growth medium where most other bacteria are incapable of reproducing, said bacterial strains are extremely efficient in removing the organic load of waste water. This is usually measured as total COD, which means the total chemical oxygen consumption (mg O_2/l). The isolated bacterial strains can particularly decompose compounds that do not decompose easily, such as chlorophenoles, polycyclic aromatic hydrocarbons (PAH compounds) and oils. They also remove heavy metals. The scope of the invention also encompasses progeny of said strains, referring to progeny of said strains that have substantially the same waste water treatment capacity as the deposited strains.

The bacteria *Bacillus* sp. DT-1, *Pseudomonas azelaica* DT-2 and *Rhizobium* sp. DT-5 further tend to flocculate, in which case they form a so-called bionetwork, which comprises lumps comprising microorganisms and other particles and which promotes the purification.

Particularly good waste water treatment results are achieved when biological water purification utilizes a bacterial mixed population comprising one or more bacteria selected from a group comprising the bacteria *Bacillus* sp. DT-1, *Pseudomonas azelaica* DT-2 and *Rhizobium* sp. DT-5, and progeny thereof. The best purification results are achieved when a mixed population is used which comprises all three bacterial strains and/or progeny thereof. In addition to these three strains, the bacterial mixed population may further comprise other microorganism strains that are useful in water treatment and that have a favourable combined effect on the purification capacity.

The best purification results are achieved when the microorganism strains DT-1, DT-2, and/or DT-5 are used together with one or more bacterial strains from the group *Pseudomonas azelaica* DT-6 having the deposit number DSM 13516, *Azospirillium* sp. DT-10 having the deposit number DSM 13517, *Ancylobacter aquaticus* DT-12 having the deposit number DSM 13518, and *Xanthobacter* sp. DT-13 having the deposit number DSM 13519, and progeny thereof. Said four strains were isolated from the biofilm of the last unit of a four cascade bioreactor for treating water containing a mixture of soaps. They can be grown in the same growth medium and under the same conditions as DT-1, DT-2 and DT-5. DT-6, DT-10, DT-12 and DT-13 improve the immobilization properties of the biofilm to supporting matrices when they are mixed with strains DT-1, DT-2 and DT-5. Association of the strains also improves the treatment process of waste water as a result of more tolerance of the biofilm formed against poisonous substances.

Bacillus sp. DT-1 is a rod which is about 1.0 - 1.2 μm in width and 3.0 - 6.0 μm in length. Partial sequencing of the 16S rDNA shows a similarity of 99.3% to *B. cereus* and 100% to *B. thuringiensis*. In identification tests DT-1 reacted as indicated below:

Anaerobic growth	+
VP reaction	+
pH in VP broth	4.8
Growth in medium pH 5.7	+
2% NaCl	+
5%	+
7%	-
10%	-
Lysozyme broth	+
Acid from	
L-arabinose	-
D-xylose	-
D-mannitol	-
D-fructose	+
Lecithinase	+
Hydrolysis of:	
casein	+

Tween 80 aesculin	weak +
Use of propionate	-
Indol reaction	-
Phenylalanine deaminase	+
Hemolysis	+
Growth in penicillin 900U/ml	+

Pseudomonas azelaica DT-2 is a rod which is 0.5 - 0.7 μm in width and 1.5 - 3.0 μm in length with 1 - 3 polar flagella and lacking fluorescent pigments. The partial sequencing of the 16S rDNA is 99.8% similar to *Ps. azelaica*. It reacts as follows:

Lysis by 3% KOH	+
Aminopeptidase (Cerny)	+
Lecithinase	-
Utilization of arabinose adipat mannitol gluconat caprat	 - + - + +

Rhizobium sp. DT-5 is a rod which is 0.5 - 0.7 μm in width and 1.5 - 3.0 μm in length. Partial 16S rDNA sequencing shows a 98.6% similarity to *R. giardinii* and 98.6% similarity to *Phyllobacterium myrisinacearum*. Physiological test results are given below. They do not confirm any of these genera.

Lysis by 3% KOH	+
Aminopeptidase (Cerny)	+
Anaerobic growth	-
Simmons citrate	+

Utilization of	
arabinose	+
mannose	+
mannitol	+
adipat	-

Other morphological, physiological and biochemical characteristics of bacterial strains DT-1, DT-2 and DT-5 are shown in Table 1.

Table 1. Morphological, physiological and biochemical characteristics of the bacterial strains.

Characteristic	Strain reaction		
	DT-1	DT-2	DT-5
Cell morphology	Straight or slightly curved rod	Straight rod	Rod
Motility	+	+	+
Formation of endospores	+	-	-
Spore form	E	-	-
Spore position	T	-	-
Expanded sporangium	-	-	-
Gram's stain	P	N	N
Catalase	+	+	+
Oxidase	+	+	+
Reduction of nitrate to nitrite	+	+	-
Denitrification	-	+	-
Argininedihydrolase	+	+	-
Hydrolysis:			
- starch	+	-	-
- gelatin	+	-	-
- acetamide	-	-	+
Urease	-	-	+
Splitting up aromatic ring	-	Orto	-

Growth at temperature of:			
35°C	+	+	+
39°C	+	+	-
40°C	+	-	-
41°C	+	-	-
43°C	-	-	-
Utilization of:			
Acetate	+	+	+
D-Alanine	-	+	-
L-Alanine	-	+	+
ß-Alanine	-	+	-
L-Arginine	+	+	+
L-Asparagine	±	+	±
L-Aspartate	±	+	-
Citrate	+	+	-
L-Cystein	-	-	+
L-Cystin	-	-	-
Ethanol	-	+	-
D-glucose	+	+	+
Glutamate	+	+	±
Glycerol	+	-	-
Glycine	-	-	-
L-Histidine	-	+	+
p-Hydroxybenzoate	-	+	-
meso-inositol	-	-	+
Lactose	-	-	-
L-Leucine	±	+	+
L-Lysine	±	+	-
Malat	+	+	-
Malonate	+	-	-
Methanol	-	-	-
L-Methionine	-	-	-
L-Proline	-	+	+
DL-Serine	+	-	-
Succinate	+	+	+

Saccharose	±	-	+
DL-Threonine	-	-	-
D-Trehalose	+	-	+
DL-Tryptophan	±	-	-
L-Tyrosine	-	+	±

P = positive

N = negative

E = of elliptical shape

T = terminal

Furthermore, the profiles of the fatty acids of bacterial strains DT-1, DT-2 and DT-5 were determined and they are shown in Figures 2 to 4. The bacteria were grown 24 hours at 28°C on tryptic soy broth agar and methyl esters were prepared for the fatty acid analysis of the whole cell, as described in publication Structure and composition of biological slimes on paper and board machines. Appl. Environ, Microbiol. 60:641-653 by Väisänen, O.M., E-L. Nurmiaho-Lassila, S.A. Marmo and M.S. Salkinoja-Salonen (1994). An aerobic TSBA library, version 3.9 (MIDI Inc., Newark, DE, USA), was used. The retention time (in minutes) is shown on the x-axis of Figures 2a and 3a, and the intensity of a peak is shown on the y-axis of the same figures. The corresponding prints of the fatty acid analyses are shown in Figures 2b, 3b and 4. The profile of the fatty acids of DT-1 is typical of the *B. cereus* group. The profile of DT-2 is typical of the RNA group I of the pseudomonads, and the profile of DT-5 points to the *Rhizobium* group.

Pseudomonas azelaica DT-6 is a 0.5 - 0.7 µm wide and 1.5 - 3.0 µm long gram-negative motile rod having 1 - 3 polar flagella and lacking fluorescent pigments. Its fatty acid analysis print (Figure 5) is typical of the RNA group I of the pseudomonads. The partial sequencing of the 16S rDNA shows a 99.8% similarity to *Ps. azelaica*. DT-6 has the following physiological reactions:

Lysis by 3% KOH	+
Aminopeptidase (Cerny)	+
Oxidase	+
Catalase	+
ADH	+

NO_2 from NO_3	+
Denitrification	weak
Urease	-
Hydrolysis of gelatin	-
Lecithinase	-
Utilization of (API 20NE) glucose arabinose adipat malat mannitol gluconat caprat	 + - + + - + +

Azospirillum sp. DT-10 is a 0.8 - 1.2 µm wide and 2.0 - 4.0 µm long gram-negative rod. Its fatty acid analyisis print (Figure 6) is typical of the α-subgroup of the proteobacteria and points to the genus *Azospirillum*. The partial sequencing of the 16S rDNA shows similarities between 92% and 97.4% to different members of the genus *Azospirillum*. The highest similarity 97.4% was found to *Azospirillum lipoferum*. The physiological reactions of DT-10 are shown below. They point to the genus *Azospirillum* but are not typical of *A. lipoferum*. DT-10 is possibly a new species of this genus.

Lysis by 3% KOH	weak
Aminopeptidase (Cerny)	+
Oxidase	+
Catalase	+
NO_2 from NO_3	+
Urease	+
ADH	-
Hydrolysis of gelatin esculin	 - -
Utilization of (sole carbon source) glucose arabinose	 - -

adipat	-
malat	+
mannitol	-
phelyacetat	-
citrate	-
caprat	-
gluconat	-
maltose	-
n-acetylglucosamin	-
α-ketoglutarate	+
sucrose	-
m-inositol	-
D-fructose	+
rhamnose	-
arabitol	-
ribose	-
Growth at 41°C	-
with 3% NaCl	-

Ancylobacter aquaticus DT-12 is a gram-negative curved rod which is 0.5 - 0.7 μm in width and 1.5 - 2.0 μm in length. The partial sequence of the 16S rDNA shows a similarity of 98.8% to *Ancylobacter aquaticus*. *Thiobacillus novellus* shows a similarity of 97.8%. The fatty acids (Figure 7) point to the α-proteobacteria. The physiological tests as shown below clearly identify the species *Ancylobacter aquaticus*.

Lysis by 3% KOH	weak
Aminopeptidase (Cerny)	+
Oxidase	+
Catalase	+
ADH	-
Urease	-
Hydrolysis of gelatin esculin	- +
NO_2 from NO_3	-
Denitrification (24 h)	-
Utilization of glucose citrate arabinose mannose mannitol maltose N-acetylglucosmin gluconat malat phenylacetat methanol formiate	 + (weak) + + - + - - - + - + weak

Xanthobacter sp. DT-13 is an irregular, motile, gram-negative rod which is 0.8 - 1.0 μm in width and 1.5 - 3.0 μm in length. The partial sequences of the 16S rDNA show similarities of 98.5% to 99.3% to different members of the genus *Xanthobacter*. *X. falvus* shows the highest similarity (99.3%). The profile of the fatty acids is typical of the subclass of α-proteobacteria. The physiological tests are not able to distinguish reliably between the species of this genus (i.e. no pigment production detected, no slime production, etc.). The physiological data are given below:

Lysis by 3% KOH	+
Aminopeptidase (Cerny)	+
Oxidase	+
Catalase	+
ADH	-
Urease (24 h)	-
Hydrolysis of gelatin esculine	- -
NO_3 utilization	-
Utilization of phenylacetate citrate malate arabinose mannose mannit caprat maltose adipate malonate methanol m-inosit m-tartrate D-gluconate phelylalanine	 - - + - - - - - + + - - + + -

The above-described bacteria are suited for use in purifying waste water. The bacteria can then be first grown in a minimal salt medium (KSN) in a shaker. Soy pepton (0.5 g/l), trypton (0.1 g/l), glucose (0.2 g/l) and potassium acetate (0.3 g/l) may be added, if desired. The growing temperature of the bacteria is about 20 - 30°C. After this, the volume of the culture is then increased in order to produce the necessary biomass for purifying the water. This stage no longer needs to be conducted under sterile conditions, in which case tap water wherein about 0.5 - 4 g/l of soap has been added can be used as the growth medium. The soap used is preferably a mixture containing ani-

onic, cationic, amphoteric and non-ionic tensides. It is preferable to use a mixture of different soaps, such as cleaning agents, fabric conditioners and detergents for clothes and dishes. The bacteria are grown as a submerged culture with air pumped thereto. The biomass can be produced as a batch culture, but preferably, it is produced as a continuous culture, or chemostat culture. It is preferable to use a carrier in the production of the biomass. Any common carrier, for example a plastic one, is suitable for this purpose. The produced biomass is then transferred into a water treatment reactor, into which the water to be purified is conveyed. A carrier for the bacteria is also used in the reactor, the carrier preferably being the same as used in the production of the biomass. The carrier is preferably one having a specific density lower than 1 g/cm^3. The carrier is generally held in place in a tank by means of a net ('fixed carrier'), for example, but sometimes the carrier is allowed to float freely in the tank ('swimming carrier').

The method of the invention is suited particularly to purifying seep water of a dump, which is here described in closer detail with reference to Figure 1. A dump is usually surrounded by a ditch to collect the seep water. Seep water refers to water seeping from a dump due to rain and ground water. This seep water containing both surface water and cavity water is usually first conveyed to a tank wherefrom the water is conveyed through a purification process before being discharged into the environment. The seep water obtained both from deep and shallow ground is preferably first conveyed to a settlement basin, from which the water is filtered through an inlet pipe 1 to a filtrate well 2, and from there, through a transfer pipe 8 to a bioreactor 3 containing said bacteria and a carrier 5. The bacteria form a so-called biofilm around the carrier. The carrier with its bacteria is usually kept below the surface of the water by means of a net. The bioreactor preferably comprises one or more separating walls 6 arranged to force the water to circulate in the reactor. The separating walls may be arranged on opposite walls, for example, as shown in Figure 1. The reactor usually further comprises an aerator 9 for conveying air into the reactor through an aeration pipe 4. The bioreactor further comprises an outlet pipe 7, through which processed water is discharged from the reactor.

In addition to purifying seep water, the present invention is extremely well suited also to purifying household and industrial grey water. Grey water refers to waste water other than that originating from lavatories, e.g. water from showers, handbasins, bath tubs and laundry rooms. The purifica-

tion method of the invention is also suited to purifying waste water from lavatories, which is called black water. The method of the invention can also be used to purify laundry and industrial waste water, which often contains a large amount of organic waste, such as oil, polycyclic aromatic hydrocarbons (PAH compounds) and/or heavy metals. The method is also suitable for purifying waste water originating from food industry and water in swimming pools.

Example 1

Production of biomass and start of a bioreactor

Bacillus sp. DT-1, *Pseudomonas azelaica* DT-2 and *Rhizobium* sp. DT-5 were each transferred to 200 ml of sterilized minimum salt medium (KSN) of the following composition (g/l of distilled water): $K_2HPO_4 \times 3H_2O$ - 1.0, $NaH_2PO_4 \times 2H_2O$ - 0.25, $(NH_4)_2SO_4$ - 0.1, $MgSO_4 \times 7H_2O$ - 0.04, $Ca(NO_3)_2 \times 4H_2O$ - 0.01, yeast extract - 0.05, pH 7.0 - 7.3, and soap mixture about 1 g/l. The soap mixture contained about equal amounts of the following detergents: laundry soap, Comfort, Cleani Family -fabric conditioner, Cleani Color, Serto Ultra, Bio Luvil, Ariel Futur, Omo Color, Tend Color, Tend Mega, Tend Total and Eko Kompakt (about 1g/l in total). The bacteria were grown in a shaker (150 - 200 rpm), at 28°C.

When the growth was dense, all three cultures were brought to one 500-litre fermenter in order to produce the necessary biomass. The fermenter contained unsterilized tap water and a total of 4 g/l of the aforementioned soap mixture, and a plastic carrier containing polyethene and having a specific density of about 0.8 g/cm^3. The carrier was kept below the surface of the liquid by means of a net. The cultivation now continued under non-sterile conditions to a turbidity of about 2 (600 nm), and then as a chemostat culture. A first inoculum obtained from the fermenter was then introduced into a bioreactor (6 m^3) according to Figure 1, diluted 1:10. The bioreactor contained seep water from a municipal dump which was first collected into a tank, wherefrom it was then transferred to a settlement basin for removal of solid matter and next, to a filtrate well, wherefrom it was pumped to the bioreactor. In principle, the system works by gravity, the only necessary pump being a submersible pump in the filtrate well. The bioreactor contained the same carrier as the fermenter used for producing the biomass. The carrier was kept below the liquid level by means of a net. The bacteria flocculated at the end of the bioreactor. The purification process was continuous, operating at a capacity of about 100 m^3/24

hours. Air was pumped so as to keep the oxygen content of the water to be processed > 7 mg/l.

Example 2

Purification of seep water

A bioreactor arranged according to Example 1 was used for purification of seep water from a municipal dump. The average COD of the waste water to be purified was about 800 mg - 6 g O_2/l. The waste water contained chlorophenoles, PAH compounds and oil, for example. The removal of these subsctances from the waste water was monitored. According to Nordtest's technical report no. 329 (accepted 9603), the compounds were defined by a gas chromatograph equipped with a mass-selective detector. The results are shown in Table 2.

Table 2

Detection	Before bioreactor	After bioreactor
COD	0.8 - 6 g/l	100 - 200 mg/l
chlorophenoles	> 1 mg/l	< 1 µg/l
PAH	1 mg/l	< 1 µg/l
oil	0.2 - 1 mg/l	200 µg/l

Example 3

Purification of municipal waste water (full scale)

Waste water from a municipal waste water plant was purified both in a manner conventionally used in the plant and by the method of the invention. Conventionally, waste water was purified by first conveying the waste water into a preliminary settlement basin in order to precipitate the solids onto the bottom. The preliminary settled water was then conveyed to an aerobic treatment basin, whereto ferrous sulphate for precipitating phosphate, and polyamine for precipitating biosludge were added. Herefrom, the water was further conveyed to a secondary settlement basin. The purification system of the invention comprised five tanks whose total volume was 7.5 m^3, the tanks being interconnected in the following order: two anaerobic tanks, whereto bacteria DT-1, DT-2 and DT-5 were added without a carrier, one aerobic tank whereto a carrier was attached (by means of a net) on which the bacteria DT-

1, DT-2 and DT-5 were immobilized, and two sedimentation tanks. The temperature was 8 - 15°C. The flow rate was 7.5 m^3/24 hours of waste water. The aeration was conducted by recycling the water through the carrier. The results are shown in Table 3.

Table 3

Parameter	Before treatment	After conventional purification	After purification of the invention
BOD7 mg O_2/l	200 - 300	10 - 15	10 - 15
COD_{Cr} mg O_2/l	250 - 500	60 - 75	40 - 50
Total nitrogen mg N/l	35 - 55	15 - 25	15 - 25
Total phosphor mg P/l	5 - 10	0.6 - 1.8	0.5 - 1.8
Fec. streptococci cfu/100 ml	10^8	2×10^4 - 3×10^4	2×10^4 - 3×10^4
Thermo-tolerant coliforms cfu/100 ml	3×10^8	2×10^4 - 4×10^4	2×10^4 - 4×10^4

The purification results achieved by the method of the invention were either as good as or better than those achieved by the conventional method, and energy consumption was significantly lower. The energy consumption in treating one cubic metre of water was 0.23 kWh at the municipal waste water treatment plant, and 0.05 - 0.1 kWh when the method of the invention was used.

Example 4

Purification of household black water (full scale)

The system comprised five tanks whose total volume was 6.5 m^3, the tanks being interconnected in the following order: two anaerobic tanks without a carrier into which the DT-1, DT-2 and DT-5 were added, one aerobic tank whereto a carrier was attached on which the bacteria DT-1, DT-2 and DT-5 were immobilized, and two sedimentation tanks. The temperature was 8 - 15°C. The flow rate was 0.5 - 5 m^3 of waste water per 24 hours. The aeration

was conducted by recycling the water through the carrier. The energy consumption was 0.05 - 0.5 kWh. The results are shown in Table 4.

Table 4

Parameter	Before treatment	After treatment
BOD7 mg O_2/l	400 - 5500	3 - 20
COD_{Cr} mg O_2/l	400 - 6000	40 - 70
Total nitrogen mg N/l	100 - 300	1 - 5
Total phosphorus mg P/l	10 - 25	0.2 - 2
Fec. streptococci cfu/100 ml	10^8 - 10^9	< 20
Thermo-tolerant coliforms cfu/100 ml	10^8 - 10^9	< 20
pH	7 - 8	6.5 - 7

Example 5

Purification of industrial waste water containing soap and heavy metals (laboratory scale)

Waste water from a coating metal industry plant was purified by a system whose effective treatment part comprised six anaerobic and twelve aerobic tanks. The bacteria DT-1, DT-2 and DT-5, which were immobilized on a carrier attached by nets, were added to all anaerobic and aerobic tanks. Each tank held 2 l. The entire system comprised 23 tanks whose total volume was 70 l, the tanks being interconnected in the following order: six anaerobic tanks (effective treatment volume), one sedimentation tank, six aerobic tanks (effective treatment volume), one sedimentation tank, six aerobic tanks (effective treatment volume), and two tanks for calcium chloride and sodium hydroxide treatments to precipitate the biomass and heavy metals. Before the treatment, the original waste water was diluted five times by gray water. After the dilution, mineral salts were added as follows: NH^{4+} 2 - 10 mg/l, NO^{3-} 5 - 20 mg/l, Mg^{2+} 2 - 10 mg/l, Ca^{2+} 0.5 - 2 mg/l, SO_4^{2-} 1 - 10 mg/l and PO_4^{3-} 2 - 20 mg/l. The temperature was 20 - 35°C and the flow rate 12 l of water per 24 hours. The results are shown in Table 5.

Table 5

Parameter	Before treatment	After treatment
COD_{Cr} mg O_2/l	19 000 - 21 000	100 - 400
Total phosphorus mg P/l	19 - 25	0.3 - 0.7
Aluminium	5 - 6	0.01 - 0.02
Chrome	1.3 - 1.5	0.01 - 0.02
Copper	35 - 40	0.03 - 0.1
Iron	1 - 2	0.02 - 0.07
Lead	23 - 25	0.02 - 0.09
Nickel	2 - 3	0.05 - 0.09
Zinc	30 - 60	0.003 - 0.007
pH	8 - 9	7 - 7.5

Example 6

Purification of household grey water for recycling (pilot scale)

The effective part of the system comprised three aerobic tanks whose single volume was 0.2 m^3. The entire system comprised six tanks whose total volume was 2.8 m^3, the tanks being interconnected in the following order: one tank for collecting grey water, three aerobic tanks comprising a fixed carrier on which the bacteria DT-1, DT-2 and DT-5 were immobilized (effective treatment volume), one aerobic tank without a carrier and one sedimentation tank, and, subsequently, a filtering system and a UV-light treatment system. The temperature was 20 - 35°C. The flow rate was about 1 m^3 per 24 hours. The results are shown in Table 6.

Table 6

Parameter	Before treatment	After treatment
COD_{Cr} mg O_2/l	150 - 400	15 - 35
Total nitrogen mg N/l	10 - 15	< 0.5
Total phosphorus mg P/l	5 - 10	< 0.1
Coliforms cfu/100 ml	1.4 - 2 x 10^6	0
pH	7.5 - 8.5	6.5 - 7

Example 7

Purification of grey water of a laundry for recycling (pilot scale)

The effective treatment part of the system comprised two aerobic tanks having the volume of 1 m^3, the tanks comprising a swimming carrier on which DT-1, DT-2 and DT-5 were immobilized. The entire system comprised ten tanks whose total volume was 23 m^3, the tanks being interconnected in the following order: one tank for collecting grey water, two aerobic tanks comprising a swimming carrier (effective treatment volume), one sedimentation tank, three aerobic tanks comprising a fixed carrier with its bacteria (effective treatment volume), one aerobic tank without a carrier, and two sedimentation tanks. The temperature of the water was 20 - 35°C, the flow rate 1 m^3 of waste water per 24 hours. The results are shown in Table 7.

Table 7

Parameter	Before treatment	After treatment
COD_{Cr} mg O_2/l	200 - 450	25 - 35
Total phosphorus mg P/l	1 - 2	< 0.1
pH	8.5 - 9	7 - 8

Example 8

Increase of immobilized biomass

Biomass of strains DT-1, DT-2, DT-5, DT-6, DT-10, DT-12 and DT-13 was produced and immobilized on a carrier as set forth in Example 1, and the amount of biomass on the carrier was weighed. The weight of one disc of the carrier was 72 ± 1 g. When DT-1, DT-2 and DT-5 were immobilized on the carrier, the weight of one disc of the carrier was 119 ± 13, i.e. the wet weight of the biomass was 47 ± 11 g per disc. When all seven bacterial strains were immobilized on the carrier, the weight of one disc of carrier was 172 ± 16, i.e. the wet weight of the biomass was 91 ± 16. The results show that DT-6, DT-10, DT-12 and DT-13 increased the immobilized biomass about twofold.

CLAIMS

1. A method of purifying waste water, **characterized** in that the water is biologically purified by a mixed population comprising the microorganisms *Bacillus* sp. DT-1 having the deposit number DSM 12560, *Pseudomonas azelaica* DT-2 having the deposit number DSM 12561, and *Rhizobium* sp. DT-5 having the deposit number DSM 12562, or progeny thereof.

2. A method as claimed in claim 1, **characterized** by purifying seep water, grey water, black water, industrial waste water and waste water from laundries.

3. A method as claimed in claim 1 or 2, **characterized** in that necessary biomass for the purification is produced in a non-sterilized growth medium comprising tap water and about 0.5 - 4 g/l of soap.

4. A method as claimed in claim 1, **characterized** in that the water is also purified by one or more microorganisms from the group *Pseudomonas azelaica* DT-6 having the deposit number DSM 13516, *Azospirillium* sp. DT-10 having the deposit number DSM 13517, *Ancylobacter aquaticus* DT-12 having the deposit number DSM 13518, and *Xanthobacter* sp. DT-13 having the deposit number DSM 13519, and progeny thereof.

5. *Bacillus* sp. DT-1 having the deposit number DSM 12560 and progeny thereof.

6. *Pseudomonas azelaica* DT-2 having the deposit number DSM 12561 and progeny thereof.

7. *Rhizobium* sp. DT-5 having the deposit number DSM 12562 and progeny thereof.

8. *Pseudomonas azelaica* DT-6 having the deposit number DSM 13516 and progeny thereof.

9. *Azospirillium* sp. DT-10 having the deposit number DSM 13517 and progeny thereof.

10. *Ancylobacter aquaticus* DT-12 having the deposit number DSM 13518 and progeny thereof.

11. *Xanthobacter* sp. DT-13 having the deposit number DSM 13519 and progeny thereof.

12. A bacterial mixed population, **characterized** by comprising *Bacillus* sp. DT-1 having the deposit number DSM 12560,

Pseudomonas azelaica DT-2 having the deposit number DSM 12561, and/or *Rhizobium* sp. DT-5 having the deposit number DSM 12562, and progeny thereof.

13. A bacterial mixed population as claimed in claim 12, **characterized** by further comprising *Pseudomonas azelaica* DT-6 having the deposit number DSM 13516, *Azospirillium* sp. DT-10 having the deposit number DSM 13517, *Ancylobacter aquaticus* DT-12 having the deposit number DSM 13518, and/or *Xanthobacter* sp. DT-13 having the deposit number DSM 13519, and progeny thereof.

14. Use of a bacterial mixed population as claimed in claim 12 or 13 in purifying waste water.

15. A bioreactor, **characterized** by comprising the microorganisms *Bacillus* sp. DT-1 having the deposit number DSM 12560, *Pseudomonas azelaica* DT-2 having the deposit number DSM 12561, and *Rhizobium* sp. DT-5 having the deposit number DSM 12562, or progeny thereof.

16. A bioreactor as claimed in claim 15, **characterized** by further comprising one or more microorganisms from the group *Pseudomonas azelaica* DT-6 having the deposit number DSM 13516, *Azospirillium* sp. DT-10 having the deposit number DSM 13517, *Ancylobacter aquaticus* DT-12 having the deposit number DSM 13518, and *Xanthobacter* sp. DT-13 having the deposit number DSM 13519, or progeny thereof.

17. A bioreactor as claimed in claim 16, **characterized** by comprising all said seven bacterial strains.

18. A bioreactor as claimed in claim 15, **characterized** by comprising one or more separating walls arranged so as to force water to circulate in the reactor.

19. A bioreactor as claimed in claim 18, **characterized** in that the bacteria are immobilized on a plastic carrier medium whose specific density is about 0.8 g/cm^3.

Abstract of the Disclosure

A method of purifying waste water biologically by using three particularly suitable bacteria: *Bacillus* sp. DT-1, *Pseudomonas azelaica*, DT-2, and/or *Rhizobus* sp. DT-5, or mixed populations thereof. The invention further relates to the bacteria and the mixed populations and use thereof in purifying waste water. The invention further relates to a bioreactor including the bacteria.

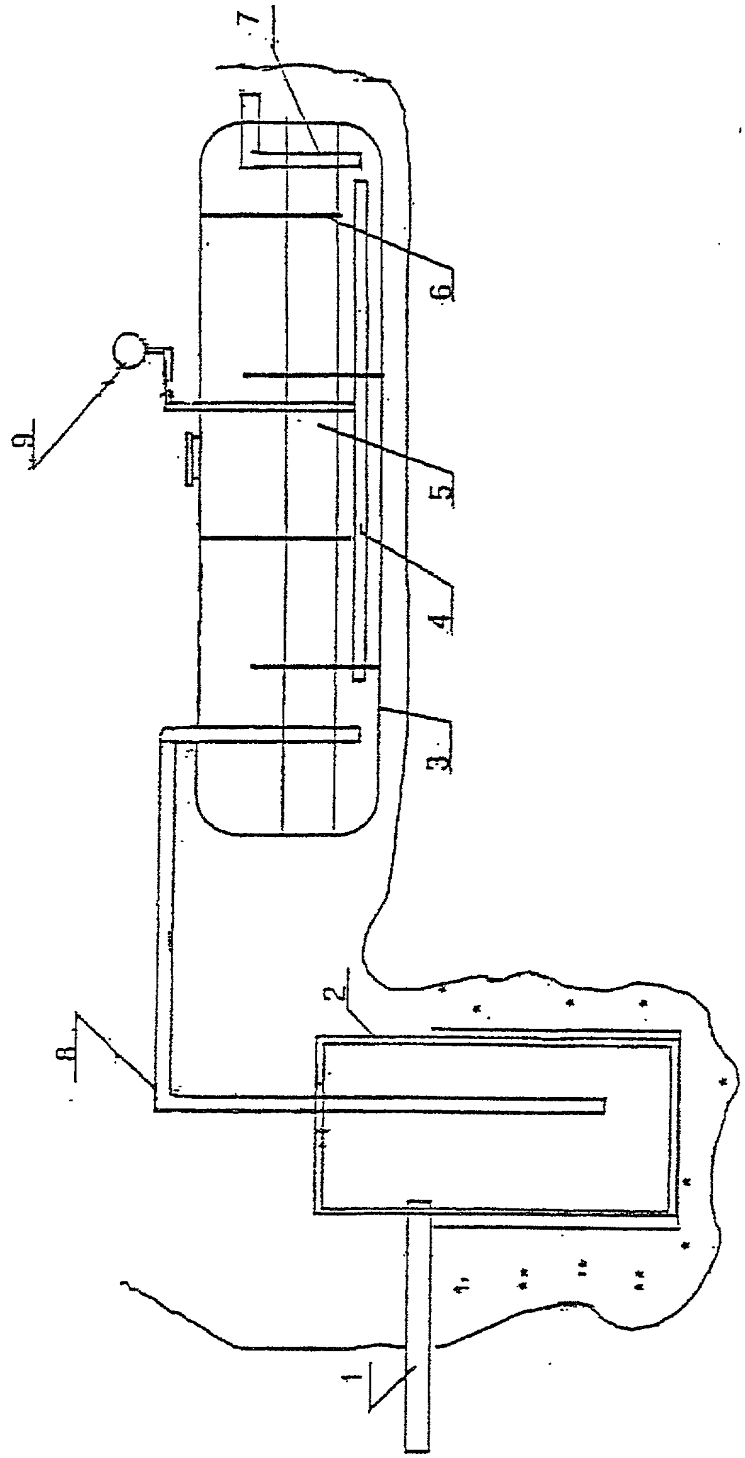

Fig. 1

Fig. 2a

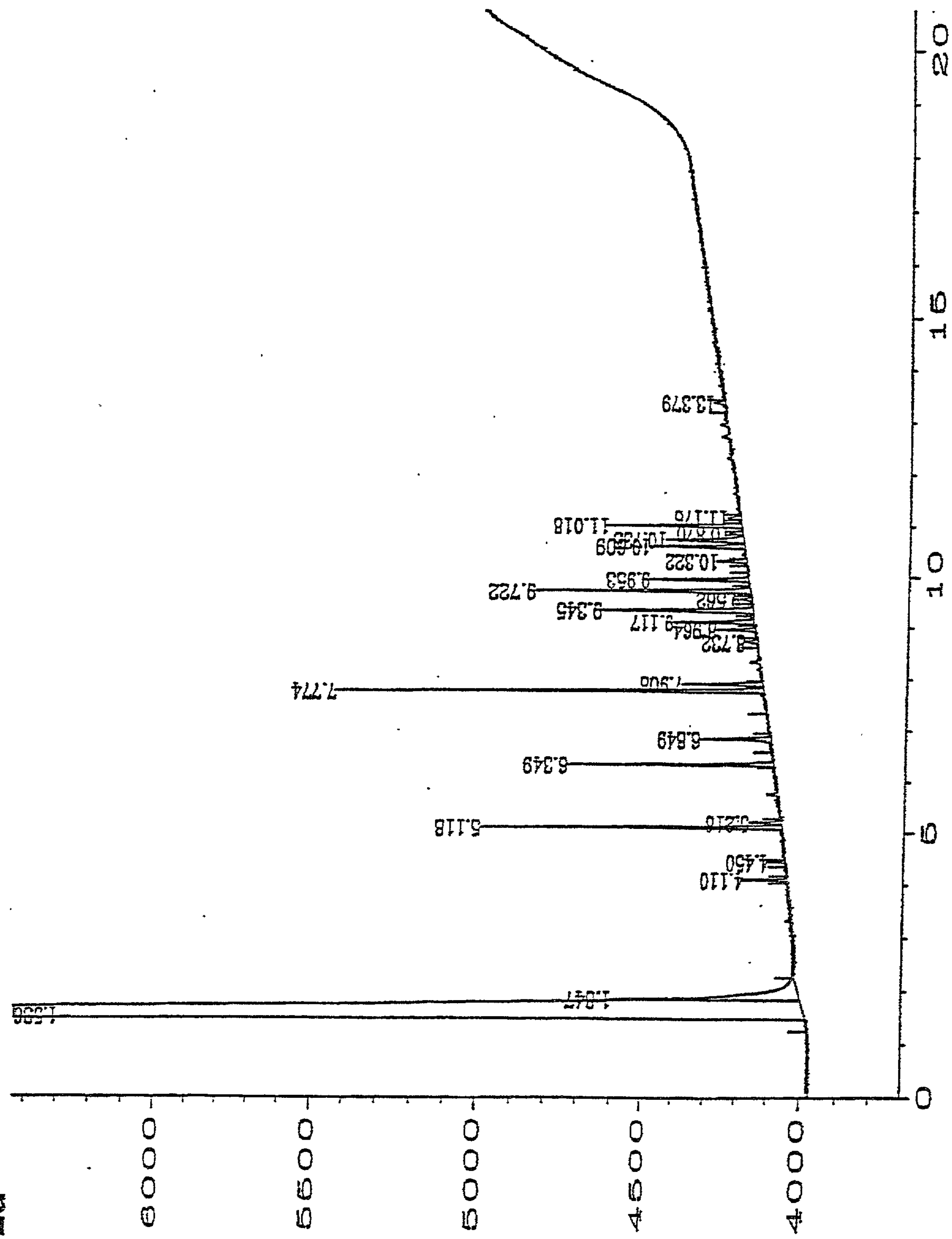

Fig. 2b

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.536	308581200	0.027	. . .	7.025	SOLVENT PEAK	. . .	< min rt	
1.847	17770	0.083	. . .	7.607	. . . .	. . .	< min rt	
4.110	1440	0.028	1.084	11.607	12:0 ISO	1.65	ECL deviates -0.001	Reference 0.003
4.450	606	0.031	1.069	12.000	12:0	0.69	ECL deviates -0.000	Reference 0.004
5.118	10566	0.030	1.047	12.612	13:0 ISO	11.73	ECL deviates 0.000	Reference 0.003
5.216	1182	0.033	1.044	12.702	13:0 ANTEISO	1.31	ECL deviates 0.001	Reference 0.004
6.349	8064	0.033	1.014	13.618	14:0 ISO	8.68	ECL deviates -0.000	Reference 0.002
6.849	3396	0.039	1.003	14.000	14:0	3.61	ECL deviates -0.000	Reference 0.002
7.774	18384	0.037	0.986	14.622	15:0 ISO	19.23	ECL deviates 0.001	Reference 0.003
7.908	3624	0.038	0.984	14.712	15:0 ANTEISO	3.78	ECL deviates 0.001	Reference 0.003
8.732	684	0.042	. . .	15.245	. . . .	. . .		
8.964	1980	0.040	0.968	15.308	16:1 w7c alcohol . .	2.03	ECL deviates 0.002	
9.117	3660	0.038	0.965	15.483	Sum In Feature 3 . .	3.75	ECL deviates 0.001	16:1 ISO I/14:0 30
9.345	8022	0.044	0.962	15.624	16:0 ISO	8.10	ECL deviates -0.002	Reference -0.001
9.562	582	0.039	0.959	15.758	16:1 w11c	0.59	ECL deviates 0.001	
9.722	10494	0.040	0.957	15.857	Sum In Feature 4 . .	10.65	ECL deviates 0.010	15:0 ISO 2OH/16:1w
9.953	4800	0.040	0.954	15.999	16:0	4.86	ECL deviates -0.001	Reference 0.001
10.322	1500	0.038	0.950	16.218	15:0 2OH	1.51	ECL deviates 0.001	
10.609	4914	0.041	0.946	16.387	ISO 17:1 w10c . . .	4.93	ECL deviates 0.000	
10.735	4188	0.043	0.945	16.462	ISO 17:1 w5c	4.20	ECL deviates 0.001	
10.870	660	0.035	0.943	16.541	17:1 ANTEISO A . . .	0.66	ECL deviates 0.000	
11.018	6588	0.040	0.941	16.629	17:0 ISO	6.58	ECL deviates -0.000	Reference 0.002
11.176	780	0.040	0.940	16.722	17:0 ANTEISO	0.78	ECL deviates 0.000	Reference 0.002
13.379	624	0.044	0.918	18.001	18:0	0.61	ECL deviates 0.001	Reference 0.003
*******	3660	. . .	. . .	. . .	SUMMED FEATURE 3 . .	3.75	12:0 ALDE ?	unknown 10.928
*******	. . . . .	. . .	. . .	. . .	. . . .	. . .	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO
*******	10494	. . .	. . .	. . .	SUMMED FEATURE 4 . .	10.65	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w

Solvent Ar.	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
308581200	96738	96054	99.29	94302	13	0.002	0.003

TSBA [Rev 3.90] Bacillus . . . . 0.265 (Bacillus cereus group)
B. thuringiensis . . . . 0.265 (Bacillus cereus group)
B. cereus 0.160
CLIN [Rev 3.90] * NO MATCH *

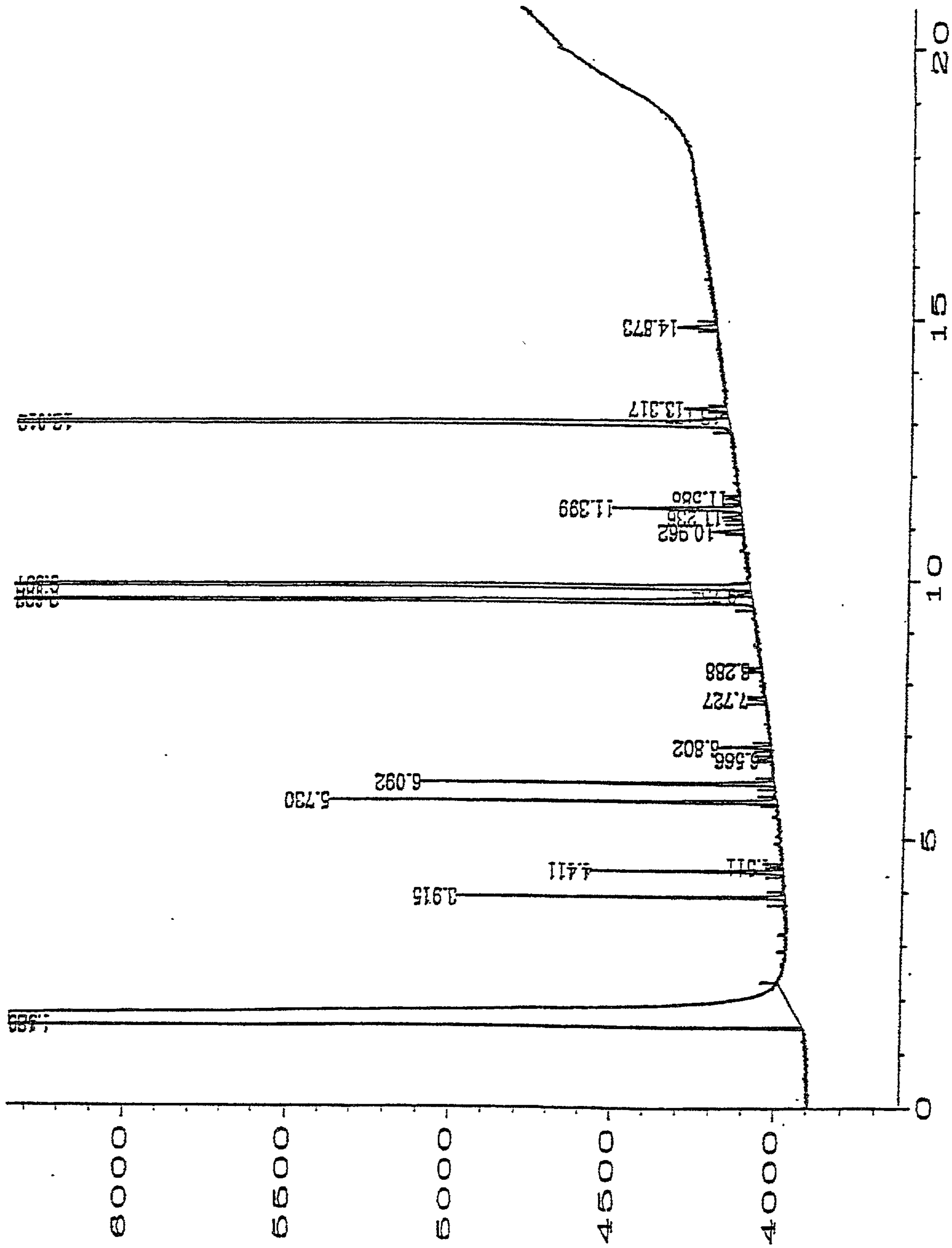
14.873
13.317
11.399
11.236
10.962
8.288
7.727
6.802
6.566
6.092
5.730
4.411
3.915
0
5
10
15
20
4000
4500
5000

Fig. 3a

Fig. 3b

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.489	150156	0.015	...	6.962	...	...	< min rt	
1.520	274442400	0.026	...	7.029	SOLVENT PEAK	...	< min rt	
3.915	10338	0.027	1.094	11.422	10:0 3OH	2.95	ECL deviates -0.001	
4.411	6870	0.030	1.071	11.999	12:0	1.92	ECL deviates -0.001	Reference 0.000
4.511	408	0.031	1.067	12.091	11:0 ISO 3OH	0.11	ECL deviates 0.001	
5.730	16770	0.032	1.028	13.177	12:0 2OH	4.50	ECL deviates -0.001	
6.092	14094	0.034	1.019	13.455	12:0 3OH	3.75	ECL deviates -0.000	
6.566	558	0.035	...	13.818	...	...		
6.802	2136	0.034	1.002	13.999	14:0	0.56	ECL deviates -0.001	Reference -0.001
7.727	846	0.041	0.984	14.624	15:0 ISO	0.22	ECL deviates 0.003	Reference 0.002
8.288	630	0.045	0.974	15.002	15:0	0.16	ECL deviates 0.002	Reference 0.002
9.603	86670	0.040	0.955	15.816	Sum In Feature 4	21.61	ECL deviates -0.001	16:1 w7c/15 iso 2OH
9.754	720	0.037	0.953	15.909	16:1 w5c	0.18	ECL deviates 0.001	
9.897	85084	0.040	0.951	15.998	16:0	21.32	ECL deviates -0.002	Reference -0.002
10.962	1596	0.042	0.938	16.629	17:0 ISO	0.39	ECL deviates -0.000	Reference -0.001
11.236	984	0.043	0.935	16.791	17:1 w8c	0.24	ECL deviates -0.001	
11.399	6552	0.044	0.933	16.887	17:0 CYCLO	1.60	ECL deviates -0.001	Reference -0.001
11.586	744	0.045	0.931	16.998	17:0	0.18	ECL deviates -0.002	Reference -0.002
13.012	163326	0.045	0.918	17.824	Sum In Feature 7	39.14	ECL deviates -0.001	18:1 w9c/w12t/w7c
13.177	570	0.054	0.916	17.919	18:1 w5c	0.14	ECL deviates 0.000	
13.317	2106	0.042	0.915	18.001	18:0	0.50	ECL deviates 0.001	Reference 0.000
14.873	2220	0.046	0.904	18.901	19:0 CYCLO w8c	0.52	ECL deviates 0.001	Reference 0.000
*******	86670	...	...	...	SUMMED FEATURE 4	21.61	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
*******	163326	...	...	...	SUMMED FEATURE 7	39.14	18:1 w7c/w9t/w12t	18:1 w9c/w12t/w7c
*******	...	...	...	...	...	...	18:1 w12t/w9t/w7c	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
274442400	404022	403464	99.86	382931	10	0.001	0.002

TSBA [Rev 3.90]	Pseudomonas	0.700	
	P. aeruginosa	0.700	
	Flavimonas	0.477	(Pseudomonas VE2)
	F. oryzihabitans	0.477	(Pseudomonas VE2)
	Chryseomonas	0.387	(Pseudomonas VE1)
	C. luteola	0.307	(Pseudomonas VE1)
CLIN [Rev 3.90]	Pseudomonas	0.339	
	P. aeruginosa*	0.339	
	P. stutzeri	0.242	
	Chryseomonas	0.322	("Pseudomonas VE1")
	C. luteola	0.322	("Pseudomonas VE1")
	Flavimonas	0.205	(Pseudomonas VE2)
	F. oryzihabitans	0.205	(Pseudomonas VE2)

Fig. 4

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.665	243677184	0.032	. . .	7.032	SOLVENT PEAK	. . .	< min rt	
1.946	536	0.030	. . .	7.563	. . . .	. . .	< min rt	
10.502	1080	0.061	0.950	15.815	Sum In Feature 4 . .	0.81	ECL deviates -0.002	16:1 w7c/15 iso 2OH
10.814	9496	0.049	0.946	15.999	16:0	7.07	ECL deviates -0.001	Reference -0.001
11.924	6120	0.051	0.934	16.631	17:0 ISO	4.50	ECL deviates 0.002	Reference 0.002
13.653	3040	0.058	. . .	17.606	. . . .	. . .		
14.043	119192	0.051	0.916	17.825	Sum In Feature 7 . .	85.92	ECL deviates -0.000	18:1 w9c/w12t/w7c
14.354	2376	0.050	0.914	17.999	18:0	1.71	ECL deviates -0.001	Reference -0.001
14.498	4160	0.055	. . .	18.081	. . . .	. . .		
14.615	2272	0.088	. . .	18.147	. . . .	. . .		
*******	1080	. . .	. . . .	. . .	SUMMED FEATURE 4 . .	0.81	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
*******	119192	. . .	. . . .	. . .	SUMMED FEATURE 7 . .	85.92	18:1 w7c/w9t/w12t	18:1 w9c/w12t/w7c
*******	. . . . . .	. . .	. . . .	. . .	. . . .	. . .	18:1 w12t/w9t/w7c	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
243677184	147736	138364	93.59	127054	3	0.001	0.001

TSBA [Rev 3.90]	Paracoccus	0.335	
	P. denitrificans	0.335	
	Bradyrhizobium	0.313	(4D, Rhiz X medium)
	B. japonicum	0.313	(4D, Rhiz X medium)
	B. j. GC subgroup A	0.313	(4D, Rhiz X medium)
	Methylobacterium	0.295	(48h, Pseudomonas mesophilica)
	M. mesophilicum*	0.295	(48h, Pseudomonas mesophilica)
	M. radiotolerans	0.248	(48h, Pseudomonas radiora)
	M. zatmanii	0.186	(48h)
CLIN [Rev 3.90]	Ochrobactrum	0.233	
	O. anthropi*	0.233	
	Sphingomonas	0.168	(Pseudomonas paucimobilis)
	S. paucimobilis	0.168	(Pseudomonas paucimobilis)

Fig. 5

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.665	239780224	0.032	. . .	7.033	SOLVENT PEAK	. . . .	< min rt	
1.947	544	0.028	. . .	7.566	. . . .	. . .	< min rt	
2.094	560	0.027	. . . .	7.844	. . . .	. . .	< min rt	
4.369	16792	0.034	1.095	11.420	10:0 3OH	4.14	ECL deviates -0.003	
4.870	2080	0.041	. . .	11.943	. . . .	. . .		
4.925	6464	0.038	1.071	12.000	12:0	1.56	ECL deviates -0.000	Reference 0.000
5.235	1624	0.053	. . .	12.259	. . . .	. . .		
6.370	23080	0.041	1.026	13.176	12:0 2OH	5.34	ECL deviates -0.002	
6.764	15360	0.041	1.016	13.455	12:0 3OH	3.52	ECL deviates -0.000	
7.535	3656	0.043	0.998	14.000	14:0	0.82	ECL deviates 0.000	Reference 0.000
10.509	109552	0.048	0.950	15.819	Sum In Feature 4 . .	23.46	ECL deviates 0.002	16:1 w7c/15 iso 2OH
10.817	102120	0.048	0.946	16.001	16:0	21.78	ECL deviates 0.001	Reference 0.001
11.045	10608	0.053	0.943	16.130	15:0 ISO 3OH	2.26	ECL deviates -0.005	
11.330	10112	0.049	. . .	16.242	. . . .	. . .		
12.376	6256	0.052	0.929	16.888	17:0 CYCLO	1.31	ECL deviates -0.000	Reference -0.000
14.045	169864	0.051	0.916	17.825	Sum In Feature 7 . .	35.07	ECL deviates 0.000	18:1 w9c/w12t/w7c
14.355	1488	0.054	0.914	17.999	18:0	0.31	ECL deviates -0.001	Reference 0.000
14.603	16104	0.057	. . .	18.139	. . . .	. . .		
15.951	2192	0.061	0.906	18.901	19:0 CYCLO w8c . . .	0.45	ECL deviates 0.001	Reference 0.002
*****	109552	. . .	. . .	. . .	SUMMED FEATURE 4 . .	23.46	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
*****	169864	. . .	. . .	. . .	SUMMED FEATURE 7 . .	35.07	18:1 w7c/w9t/w12t	18:1 w9c/w12t/w7c
*****	. . . . .	. . .	. . .	. . .	. . . .	. . .	18:1 w12t/w9t/w7c	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
39780224	497352	467432	93.98	443648	6	0.002	0.001

TSBA [Rev 3.90] Pseudomonas 0.457
P. aeruginosa 0.457
CLIN [Rev 3.90] Pseudomonas 0.226
P. aeruginosa* 0.226
P. stutzeri 0.147
P. putida 0.119
P. p. biotype A* 0.119
Chryseomonas . . . . 0.153 ("Pseudomonas VE1")
C. luteola . . . . 0.153 ("Pseudomonas VE1")

Fig. 6

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.660	248210304	0.031	. . .	7.034	SOLVENT PEAK	. . .	< min rt	
1.857	920	0.025	. . .	7.407	. . . .	. . . .	< min rt	
1.939	1296	0.029	. . .	7.562	. . . .	. . .	< min rt	
9.919	1216	0.049	0.957	15.486	Sum In Feature 3 . .	4.89	ECL deviates 0.004	16:1 ISO I/14:0 3OH
10.480	3256	0.048	0.950	15.816	Sum In Feature 4 . .	13.00	ECL deviates -0.001	16:1 w7c/15 iso 2OH
10.789	1712	0.051	0.947	15.999	16:0	6.81	ECL deviates -0.001	Reference -0.003
13.469	1120	0.058	0.924	17.517	16:0 3OH	4.35	ECL deviates -0.003	
14.009	16984	0.050	0.920	17.821	Sum In Feature 7 . .	65.69	ECL deviates -0.001	18:1 w7c/w9t/w12t
16.249	1376	0.065	0.910	19.089	18:1 2OH	5.26	ECL deviates 0.001	
*******	1216	. . .	. . .	. . .	SUMMED FEATURE 3 . .	4.89	12:0 ALDE ?	unknown 10.928
*******	. . . . . .	. . .	. . .	. . .	. . . .	. . .	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
*******	3256	. . .	. . .	. . .	SUMMED FEATURE 4 . .	13.00	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
*******	16984	. . .	. . .	. . .	SUMMED FEATURE 7 . .	65.69	18:1 w7c/w9t/w12t	18:1 w9c/w12t/w7c
*******	. . . . . .	. . .	. . .	. . .	. . . .	. . .	18:1 w12t/w9t/w7c	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
248210304	25664	25664	100.00	23797	1	0.002	0.003

QUESTION ANALYSIS: TOTAL AREA LESS THAN 50000. CONCENTRATE AND RE-RUN.

TSBA [Rev 3.90] Azospirillum 0.786

A. brasilense 0.786

CLIN [Rev 3.90] Roseomonas 0.599

R. fauriae** 0.599

Fig. 7

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.664	241080192	0.032	. . .	7.031	SOLVENT PEAK	. . .	< min rt	
10.814	8176	0.048	0.946	16.000	16:0	6.80	ECL deviates 0.000	Reference -0.001
14.041	102160	0.052	0.916	17.824	Sum In Feature 7 . .	83.22	ECL deviates -0.001	18:1 w9c/w12t/w7c
14.352	2552	0.054	0.914	17.999	18:0	2.07	ECL deviates -0.001	Reference -0.002
14.608	1544	0.077	. . .	18.143	. . . .	. . .		
15.949	9720	0.053	0.906	18.901	19:0 CYCLO w8c . . .	7.83	ECL deviates 0.001	Reference 0.001
*******	102160	. . .	. . .	. . .	SUMMED FEATURE 7 . .	83.22	18:1 w7c/w9t/w12t	18:1 w9c/w12t/w7c
*******	. . . . .	. . .	. . .	. . .	. . . .	. . .	18:1 w12t/w9t/w7c	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
241080192	124152	122608	98.76	112432	3	0.001	0.001

```
TSBA [Rev 3.90] Ochrobactrum . . . . . . . . . . . . . . . . . . . . . 0.620 (Achromobacter Vd, CDC group Vd)
                 O. anthropi . . . . . . . . . . . . . . . . . . . . . 0.620 (Achromobacter Vd, CDC group Vd)
                Bradyrhizobium . . . . . . . . . . . . . . . . . . . . 0.587 (4D, Rhiz X medium)
                 B. japonicum . . . . . . . . . . . . . . . . . . . . . 0.587 (4D, Rhiz X medium)
                  B. j. GC subgroup A . . . . . . . . . . . . . . . . . 0.587 (4D, Rhiz X medium)
                Xanthobacter . . . . . . . . . . . . . . . . . . . . . 0.500
                 X. agilis . . . . . . . . . . . . . . . . . . . . . . 0.500
                 X. flavus . . . . . . . . . . . . . . . . . . . . . . 0.254
CLIN [Rev 3.90] Ochrobactrum . . . . . . . . . . . . . . . . . . . . . 0.548
                 O. anthropi* . . . . . . . . . . . . . . . . . . . . . 0.548
```

Fig. 8

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.664	248735360	0.031	. . .	7.031	SOLVENT PEAK	. . .	< min rt	
1.770	6064	0.024	. . .	7.231	. . . .	. . .	< min rt	
1.862	2552	0.027	. . .	7.405	. . . .	. . .	< min rt	
1.945	1096	0.034	. . .	7.562	. . . .	. . .	< min rt	
10.815	2752	0.046	0.946	15.999	16:0	2.81	ECL deviates -0.001	Reference -0.001
11.327	1448	0.054	. . .	16.291	. . . .	. . .		
12.575	1712	0.058	0.927	17.002	17:0	1.72	ECL deviates 0.002	Reference 0.001
13.500	968	0.059	0.920	17.521	16:0 3OH	0.96	ECL deviates 0.001	
14.041	90456	0.051	0.916	17.825	Sum In Feature 7 . .	89.56	ECL deviates -0.000	18:1 w9c/w12t/w7c
14.352	3920	0.049	0.914	17.999	18:0	3.87	ECL deviates -0.001	Reference -0.002
14.499	760	0.044	. . .	18.082	. . . .	. . .		
14.613	49552	0.055	. . .	18.147	. . . .	. . .		
17.575	1096	0.057	0.902	19.834	20:1 w9t	1.07	ECL deviates 0.001	
******	90456	. . .	. . .	. . .	SUMMED FEATURE 7 . .	89.56	18:1 w7c/w9t/w12t	18:1 w9c/w12t/w7c
******	. . .	. . .	. . .	. . .	. . . .	. . .	18:1 w12t/w9t/w7c	

olvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
248735360	152664	100904	66.10	92494	3	0.001	0.001

QUESTION ANALYSIS: PERCENT AREA NAMED IS LESS THAN 85. CHECK FOR CONTAMINATION.

TSBA [Rev 3.90]	Methylobacterium	0.782	(48h, Pseudomonas radiora)
	M. radiotolerans	0.782	(48h, Pseudomonas radiora)
	M. mesophilicum*	0.708	(48h, Pseudomonas mesophilica)
	M. zatmanii	0.674	(48h)
	Rhodobacter	0.657	
	R. sphaeroides	0.657	
	R. capsulatus	0.454	
	Xanthobacter	0.647	
	X. flavus	0.647	
CLIN [Rev 3.90]	Methylobacterium	0.512	
	M. mesophilicum	0.512	
	Ochrobactrum	0.403	
	O. anthropi*	0.403	

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

the specification of which: ***(check one)***

REGULAR OR DESIGN APPLICATION

[ ] is attached hereto.

[ ] was filed on as application Serial No. and was amended on (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

[x] was described and claimed in International application No. PCT/FI00/00624 filed on 6th July 2000 and as amended on 6th September 2001(if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, '1.56.

PRIORITY CLAIM

I hereby claim foreign priority benefits under 35 USC 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)

Country	Application Number	Date of Filing (day, month, year)	Priority Claimed
Finland	991595	12 July 1999	X

(Complete this part only if this is a continuing application.)

I hereby claim the benefit under 35 USC 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 USC 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations '1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing Date) (Status--patented, pending, abandoned)

POWER OF ATTORNEY

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

As a named inventor, I hereby appoint the registered patent attorneys represented by Customer No. **000466** to prosecute this application and transact all business in the Patent and Trademark Office connected therewith, including: **Robert J. PATCH, Reg. No. 17,355, Andrew J. PATCH, Reg. No. 32,925, Robert F. HARGEST, Reg. No. 25,590, Benoît CASTEL, Reg. No. 35,041, Eric JENSEN, Reg. No. 37,855, Thomas W. PERKINS, Reg. No. 33,027, and Roland E. LONG, Jr., Reg. No. 41,949,**

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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